

conservation. It has been shown that AcAOX may play a role in the energetic status of the amoeba cell (by decreasing the yield of ATP synthesis) and in attenuating reactive oxygen species production. AcAOX is stimulated by purine nucleotides, except ATP that has an inhibitory effect on AcAOX activity.

A gene for AcAOX has been identified but there has been no direct functional evidence that it encodes cyanide-resistant mitochondrial oxidase. Using AcAOX cDNA sequence we performed relative quantification real time PCR and found that changes in AcAOX mRNA levels during growth of *A. castellanii* batch culture follows the pattern of changes in AcAOX protein levels and the enzyme activity.

In order to confirm an enzymatic function of the AcAOX gene product, we performed oxygen consumption measurements of *Escherichia coli* DH5 α strain transformed with pDrive containing the AcAOX gene. In transformed bacterial cells, the cyanide-resistant benzohydroxamate-sensitive respiration was observed, indicating function of active AcAOX enzyme. Moreover, the activity was stimulated by GMP and inhibited by ATP, as it has been previously shown for AcAOX in isolated *A. castellanii* mitochondria. The AcAOX protein was also identified in transformed bacterial cells by immunodetection.

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14P4

Cytochrome *bd*-II from aerobic respiratory chain of *Escherichia coli* is a proton-motive force generator

Dmitry A. Bloch¹, Vitaliy B. Borisov², Ranjani Murali³, Marina L. Verkhovskaya¹, Huazhi Han³, Robert B. Gennis³, Michael I. Verkhovsky¹

¹Helsinki Bioenergetics Group, Institute of Biotechnology, University of Helsinki, Helsinki, Finland

²A.N.Belozersky Institute of Physico-Chemical Biology, Moscow State University "Lomonosov", Moscow, Russia

³University of Illinois at Urbana-Champaign, Urbana, IL, USA

E-mail: dmitry.bloch@helsinki.fi

Escherichia coli is known to couple aerobic respiratory catabolism to ATP synthesis by virtue of the three primary proton-motive force (*pmf*) generators: NADH dehydrogenase I (NDH-I), cytochrome *bo*₃, and cytochrome *bd*-I. The non-pumping, copper-lacking three-heme *bd*-type oxidase couples oxidation of quinol to reduction of O₂ to H₂O [1,2]. Nevertheless, *E. coli* mutant strains deficient in NDH-I, *bo*₃, and *bd*-I can grow under aerobic and glucose-limited conditions, although its sole terminal oxidase cytochrome *bd*-II was proposed to be non-electrogenic. In the present work, the ability of cytochrome *bd*-II to generate *pmf* is reexamined. Absorption and fluorescence spectroscopy and oxygen pulse methods all show that in the steady-state, cytochrome *bd*-II is capable of producing both electrical and chemical components of *pmf* yielding H⁺/e⁻ ratio of 0.94 ± 0.18; this is sufficient to drive ATP synthesis and transport of nutrients. Microsecond time-resolved, single-turnover electrometry provides evidence for a molecular mechanism of the proton motive force production [3]. The ability to induce cytochrome *bd*-II biosynthesis allows *E. coli* to remain energetically competent under a variety of environmental conditions, particularly at the extremely low oxygen pressure and carbon and phosphate starvation. Work supported by RFBR (grant 11-04-00031-a).

References

- [1] V.B. Borisov, R.B. Gennis, J. Hemp, M.I. Verkhovsky, Biochim. Biophys. Acta 1807 (2011) 1398–1413.
- [2] D.A. Bloch, V.B. Borisov, T. Mogi, M.I. Verkhovsky, Biochim. Biophys. Acta 1787 (2009) 1246–1253.

- [3] V.B. Borisov, R. Murali, M.L. Verkhovskaya, D.A. Bloch, H. Han, R.B. Gennis, M.I. Verkhovsky, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 17320–17324.

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14P5

Proton pumping in cytochrome *c* oxidase – An explicit gating mechanism based on experimental information, electrostatic considerations and QM calculations

Margareta R.A. Blomberg, Per E.M. Siegbahn

Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden

E-mail: mb@fysik.su.se

In cytochrome *c* oxidase, the terminal enzyme in the respiratory chain, protons are pumped against the electrochemical gradient over the mitochondrial membrane, to store the energy released when molecular oxygen is reduced to water. A gating mechanism for the protons, involving a positively charged transition state was suggested based on electrostatic interpretations of kinetic experiments [1–4]. The predictions from that analysis have been tested using hybrid DFT with large chemical models (about 400 atoms). The proposal that a positively charged transition state for proton transfer is needed to obtain a gate is confirmed by the QM calculations. It is shown that a few critical relative energy values from the earlier studies are reproduced with quite high accuracy using this large model. Examples are the low forward barrier for proton transfer from the N-side of the membrane to the pump-loading site when the electron transfer cofactor heme *a* is reduced, and the corresponding high back leakage barrier when heme *a* is oxidized. At present MD simulations on even larger models are performed to give new starting structures for the QM calculations and thereby further validate the calculated energetics.

References

- [1] P.E.M. Siegbahn, M.R.A. Blomberg, On the proton pumping mechanism in cytochrome *c* oxidase, J. Phys. Chem. A 112 (2008) 12772–12780.
- [2] P.E.M. Siegbahn, M.R.A. Blomberg, Quantum chemical studies of proton-coupled electron transfer in metalloenzymes, Chem. Rev. 110 (2010) 7040–7061.
- [3] M.R.A. Blomberg, P.E.M. Siegbahn, A quantum chemical study of the mechanism for proton coupled electron transfer leading to proton pumping in cytochrome *c* oxidase, Mol. Phys. 108 (2010) 2733–2743.
- [4] M.R.A. Blomberg, P.E.M. Siegbahn, The mechanism for proton pumping in cytochrome *c* oxidase from an electrostatic and quantum chemical perspective, Biochim. Biophys. Acta 1817 (2012) 495–505.

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14P6

Cytochrome *bd* oxidase: Direct observation of the catalytic intermediates at steady-state

V.B. Borisov¹, E. Forte², P. Sarti^{2,3}, A. Giuffrè³

¹Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Leninskie Gory, Moscow 119991, Russian Federation

²Department of Biochemical Sciences and Istituto Pasteur – Fondazione Cenci Bolognietti, Sapienza University of Rome, Piazzale Aldo Moro 5, I-00185 Rome, Italy

³CNR Institute of Molecular Biology and Pathology, Piazzale Aldo Moro 5, I-00185 Rome, Italy
E-mail: alessandro.giuffre@uniroma1.it

Cytochrome *bd* is a prokaryotic respiratory quinol oxidase identified in a number of pathogens, that is preferentially expressed under low O₂ tension or other “stress” conditions [1]. The enzyme couples the exergonic reduction of O₂ to 2 H₂O to proton motive force generation by transmembrane charge separation. Apart from its role in cell bioenergetics, evidence suggests that cytochrome *bd* accomplishes a number of additional functions of physiological relevance for the bacterial cell, being seemingly implicated also in microbial resistance to NO-stress (reviewed in [2]) and, in general, adaptation to the hostile conditions created by host immunity during the infection process.

Cytochrome *bd* from *Escherichia coli* contains three hemes, hemes *b*₅₅₈ and *b*₅₉₅ and heme *d*, where O₂ chemistry takes place through sequential formation of a few catalytic intermediates. Here, the isolated cytochrome *bd* was investigated by stopped-flow multi-wavelength absorption spectroscopy with the aim of measuring the occupancy of the catalytic intermediates at steady-state. We found that, under turnover conditions sustained by dithiothreitol-reduced ubiquinone and O₂, the ferryl and oxy-ferrous species are the mostly populated catalytic intermediates, with a minor fraction of the enzyme containing ferric heme *d* and possibly reduced heme *b*₅₅₈ [3]. These new findings differ from those obtained with mammalian cytochrome *c* oxidase [4], where oxygen intermediates were not found to be populated at detectable levels under similar conditions. The results are discussed in the light of previously proposed models of the cytochrome *bd* catalytic cycle.

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References

- [1] V.B. Borisov, R.B. Gennis, J. Hemp, M.I. Verkhovsky, The cytochrome *bd* respiratory oxygen reductases, *Biochim. Biophys. Acta* 1807 (2011) 1398–1413.
- [2] A. Giuffrè, V.B. Borisov, D. Mastronicola, P. Sarti, E. Forte, Cytochrome *bd* oxidase and nitric oxide: from reaction mechanisms to bacterial physiology, *FEBS Lett.* 586 (2012) 622–629.
- [3] V.B. Borisov, E. Forte, P. Sarti, A. Giuffrè, Catalytic intermediates of cytochrome *bd* terminal oxidase at steady-state: Ferryl and oxy-ferrous species dominate, *Biochim. Biophys. Acta* 1807 (2011) 503–509.
- [4] M.G. Mason, P. Nicholls, C.E. Cooper, The steady-state mechanism of cytochrome *c* oxidase: redox interactions between metal centres, *Biochem. J.* 422 (2009) 237–246.

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14P7

Investigation of the proton pump and exit pathway in cytochrome *ba*₃ from *Thermus thermophilus*

H. Chang, S.K. Choi, A. Vakkasoglu, Y. Chen, J. Hemp, J. Fee, R.B. Gennis
Department of Biochemistry and Center for Biophysics and Computational Biology, University of Illinois, Urbana, IL, USA
Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA, USA
E-mail: choi123@illinois.edu

The heme-copper oxidases are redox driven proton pumps. These enzymes catalyze the reduction of O₂ to water and use the free energy

of this reaction to generate a proton motive force. In this work we investigate the location of the proton loading site and exit pathway for pumped protons in the *ba*₃-type oxygen reductase from *Thermus thermophilus*. Previous studies suggested that one or both of the propionates of heme *a*₃ and residues in their vicinity may be important for these functions, and this was investigated by site directed mutagenesis. The data show that none of the amino acid residues near these propionates is essential for proton pumping or oxidase activity, including several residues previously postulated to be important: D372, H376 and E126^{II} (in subunit II) and Y133. However, two mutants, D372I and H376N, exhibit high catalytic turnover and normal spectral features but do not pump protons or do so with noticeably diminished stoichiometry. It is concluded that the proton loading site might be either propionate-A of heme *a*₃ or a cluster of groups centered about a conserved water molecule that is hydrogen bonded to both of the heme *a*₃ propionates as well as to one of the histidine ligands to Cu_B.

References

- [1] H.Y. Chang, *Proc. Natl. Acad. Sci. U. S. A.* 109 (2012) 5259–5264.
- [2] H.Y. Chang, *Proc. Natl. Acad. Sci. U. S. A.* 106 (2009) 16169–16173.

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14P8

Rescue of complex IV biogenesis by the cytosol-synthesized subunit II (Cox2) precursor carrying the point mutation W56R

Valentín Cruz-Torres¹, Miriam Vázquez-Acevedo¹, Rodolfo García-Villegas¹, Xochitl Pérez-Martínez¹, Guillermo Mendoza-Hernández², Diego González-Halphen¹
¹Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, México D.F., Mexico
²Depto. Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México, México D.F., Mexico
E-mail: dhalphen@ifc.unam.mx

Cytochrome *c* oxidase (complex IV or CcO) catalyzes oxygen reduction coupled to proton pumping. It is composed of 10–13 subunits and its biogenesis involves both mitochondrion- and nucleus-encoded polypeptides [1]. In yeast, as in the vast majority of eukaryotes, the three largest subunits of Cco are encoded in the mitochondrial genes COX1, COX2 and COX3. Deletion of the yeast COX2 gene results in loss of respiration (Δ cox2 strain). Supekova et al. [2] were able to restore growth of a Δ cox2 mutant in non-fermentable carbon sources after transformation with a vector expressing Cox2 with a mitochondrial targeting sequence and the point mutation W56R (Cox2W56R). In this work, the CcO carrying the allotopically-expressed Cox2W56R was studied. Isolated yeast mitochondria from the wild-type (WT) and the Δ cox2 + Cox2W56R strains were solubilized and subjected to Blue Native electrophoresis. In-gel activity of CcO and spectroscopic quantitation of cytochromes revealed that only 40% of complex IV is present in the complemented strain as compared to the wild-type strain. CcOs from the WT and the rescued mutant exhibited similar subunit composition, although activity was 20–25% lower in the enzyme containing Cox2W56R than in the one containing native Cox2 (Cox2WT). Tandem mass spectrometry confirmed that W56 was substituted by R56 in Cox2W56R. In addition, as judged by Edman degradation, Cox2W56R exhibited the same N-terminus than Cox2WT, indicating that both the MTS of Oxa1 and the leader sequence of 15 residues were removed from Cox2W56R during maturation. Thus, Cox2W56R is identical to Cox2WT except for the point mutation W56R. Mitochondrial Cox1 synthesis is strongly reduced in Δ cox2 mutants, but the